ASSESSING COLOSTRAL AND SERUM IMMUNOGLOBULIN G IN ALPACAS USING BRIX REFRACTOMETRY AND TOTAL SERUM PROTEIN

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Abstract

Introduction

Assessment of passive transfer of immunity is a critical practice implemented commonly in the dairy and equine fields. This study compared direct and indirect methods of measuring immunoglobulin G concentration in colostrum and serum of alpacas during the first week of life to determine if on-farms tools were accurate. Direct IgG concentration was measured using radial immunodiffusion assays, whilst indirect IgG concentration was measured using optical and digital Brix refractometry and total serum protein.

Objective

The main objective of this study was to determine if Brix refractometry and total serum protein were accurate compared to radial immunodiffusion when measuring colostral and serum IgG in alpacas. Radial immunodiffusion is expensive, time-consuming and poorly available compared to cheap, easily accessible alternatives like Brix refractometry and total serum protein. The study also aimed to determine if colostral and serum IgG concentrations in alpacas were correlated.

Methods

The study was a pilot trial, aimed to determine if on-farm tools were accurate and feasible prior to investigation into the reference intervals for alpaca colostral and serum IgG concentration. Alpacas were recruited from a farm in the eastern Riverina district of New South Wales and were Suri breed. Colostrum was collected from the hembra within 24 h of parturition by the owners and whole blood collected from cria by the investigators between 1 and 7 days of age. Clinical examination of each hembra and cria prior to sample collection occurred to ensure dehydration was not a contributing factor to high IgG concentration and that all were healthy. Direct IgG concentration was determined following the manufacturer's instructions on the radial immunodiffusion assay kits and was indirectly estimated using Brix refractometry for total solids and clinical refractometry for total serum protein.

Results

There was a strong correlation between optical and digital Brix refractometry, and colostral IgG concentration determined by RID with statistical significance ($p < 0.05$). Optical and digital Brix refractometers showed high sensitivity for detecting high- and low-quality colostrum, and for detecting adequate and inadequate transfer of immunity. There was a moderate correlation between serum IgG concentration determined by RID and optical and digital Brix refractometry, and serum IgG concentration determined by RID and total serum protein, with no statistical significance ($p > 0.05$). There was a moderate correlation between cria age and serum IgG concentration, although cria weight, and dam age and parity showed no significant correlation with colostral or serum IgG concentrations. A moderate correlation between colostral and serum IgG concentration was found.

Conclusion

Optical and digital Brix refractometry for colostral IgG estimation and optical and digital Brix refractometry and total serum protein for serum IgG estimation are reliable, accurate and easy-to-use tools that can be used on-farm to detect a failure of passive transfer. Further research is required to determine the reference intervals for these tools to be practical.

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Chapter 1: Introduction

The adequate transfer of passive immunity is an important factor of neonatal animal health and survivability. The transfer of immunoglobulins, particularly immunoglobulin G (IgG) across the intestinal mucosa and into the bloodstream within the first hours of life is the physiological process that underpins passive transfer of immunity (Garmendia et al., 1987). Transfer of IgG in mammals from an immune-competent dam to its neonate provides immune protection (Westrom et al. 2020). This IgG transfer can be measured indirectly, yet accurately with on-farm tools such as Brix refractometers. This is well documented in the dairy and equine industries (Korosue et al., 2012; Morrill et al., 2015; Quigley et al., 2013; Thornhill et al., 2015; Zakian et al., 2018). Alpaca owners and veterinary researchers have increasingly recognised the possible impact of inadequate immunoglobulin transfer on the ability of cria to thrive, yet no on-farm tools have been widely accessible or adequately researched for their accuracy. Previous research overseas examining alpaca IgG concentrations have presented conflicting results with respect to adequate IgG concentrations in hembra colostrum and cria blood and the appropriate threshold indicating adequate passive transfer of immunity (Pinn et al., 2013; Garmendia et al., 1987). Research in the dairy and equine fields have claimed that adequate passive transfer of immunity reduces the risk of sepsis, diarrhoea, respiratory disease and other infections in neonates (Davis & Giguère, 2005; Zakian et al., 2018), whilst improving growth rates and survivability (Elsohaby et al., 2019; Mishra et al., 2017). Dated research has found that cria are susceptible to infections and death without adequate transfer of IgG (Garmendia et al., 1987), with more recent studies presenting conflicting results on the definition of adequate in terms of serum IgG concentration (Pinn et al., 2013; Weaver, Tyler, Scott, et al., 2000). Additionally, the tools used to assess passive transfer of immunity in the dairy and equine fields are not commonly used or understood when assessing alpacas, and the research determining their efficacy and accuracy is sparse and lacking currency (Bravo et al., 1997; Garmendia et al., 1987; Weaver et al., 2000; Elsohaby et al., 2017). It is therefore clear that further research is required to

not only determine the definition of adequate serum and colostral IgG concentrations in alpacas, but to also assess whether on-farm tools are practical and accurate.

There are a variety of indirect tools and kits available for use in clinics and on-farm, including optical and digital Brix refractometry, total serum protein, serum zinc sulfate turbidity test and serum y-glutamyl transferase activity (GGT) (Zakian et al., 2018). The Brix refractometer is an inexpensive tool used to estimate colostral and serum IgG concentration by providing a percentage value that estimates the total solids of the sample. There are optical and digital options available which have been shown to have a high correlation when used to estimate serum IgG concentrations in dairy calves and foals (Bielmann et al., 2010; Elsohaby, Riley, et al., 2019). Total serum protein can be estimated using a clinical refractometer which measures the refractive index of a sample which is determined by its total solids (Morrill et al., 2015). These tools have all been extensively used and researched in the dairy and equine fields with stark differences between species, highlighting the need for further research on the accuracy and practicality of these tools in the camelid field to allow their use on farms.

The aim of the following study was to determine whether on-farm tools such as Brix refractometry and total serum protein were accurate compared to direct methods of measuring colostral and serum IgG concentration in alpacas. The study hypothesis specified that the indirect methods would be in agreeance with direct assays.

Chapter 2: Literature review

2.1 Introduction

Passive transfer of immunity involves the absorption of maternal immunoglobulins across the intestinal wall into the bloodstream of neonates, providing protection against systemic diseases until the neonate is able to develop its own active immune response (Korosue et al., 2012). A failure of passive transfer occurs when inadequate immunoglobulins are absorbed across the gastrointestinal tract of a neonate within the first days of life, predisposing to sepsis, diarrhoea and other infections (Davis & Giguère, 2005). The assessment of colostral and serum immunoglobulins, specifically immunoglobulin G (IgG) has been extensively studied in dairy calves and foals (Korosue et al., 2012; Morrill et al., 2015; Quigley et al., 2013; Thornhill et al., 2015; Zakian et al., 2018).The research in alpacas is sparse internationally (Elsohaby et al., 2017; Pinn et al., 2013; Weaver, Tyler, Scott, et al., 2000) and almost non-existent in Australia, with only a single study by Elsohaby et al. (2017) found.

2.2 Failure of passive transfer of immunity in production animals

2.2.1 Physiology of passive transfer of immunity

Colostrum, the first milk produced by the mammary gland after parturition contains maternal immunoglobulins. Similarly to other ungulate species, alpacas are born hypogammaglobulinemic (low blood IgG concentration), with a significant increase in serum IgG concentration achieved once ingestion of colostrum occurs (Westrom et al., 2020). Without this increase in serum IgG concentration, cria are susceptible to infections and death (Garmendia et al., 1987). For adequate transfer of immunity to occur colostral IgG concentration must be adequate, the neonate must ingest an adequate volume of colostrum and IgG must be efficiently absorbed (Zakian et al., 2018).

2.2.2 Recognition and intervention in calves and foals

In calves the ability of immunoglobulins to pass through the wall of the small intestine into the bloodstream diminishes by 24-36 hours of age (Weaver, Tyler, VanMetre, et al., 2000; Westrom et al., 2020). Failure of passive transfer occurs when the calf fails to ingest or absorb adequate immunoglobulins within this period, making it susceptible to infection. This can lead to increased mortality and morbidity in neonates (Figure 2.1), often resulting in increased severity of diarrhoea and respiratory diseases in dairy calves (Zakian et al., 2018).

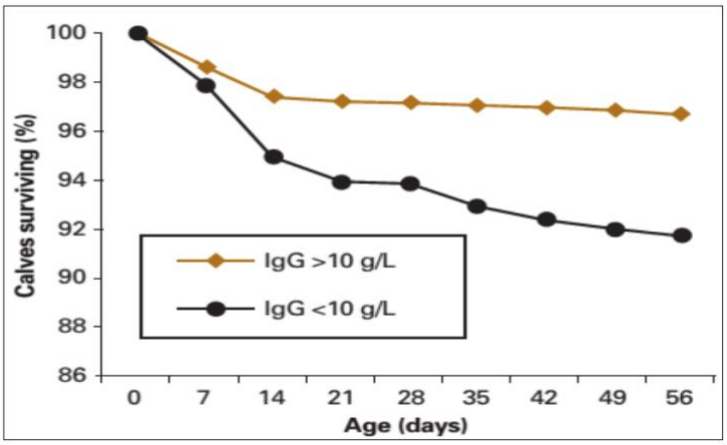

Figure 2.1 A graphical representation of calf survival rate with adequate serum IgG concentration compared to calf survival rate with inadequate serum IgG concentration, (taken from Mishra et al. (2017). In calves with serum IgG concentration below 10 g/L survival is reduced.

In horses, the time in which immunoglobulins can no longer enter the bloodstream from the intestinal tract is 24 hours of age, with transfer diminishing from 12 hours (Smith, 2009). Early diagnosis of a failure of passive transfer is important as supplemental colostrum can be provided within 12 hours of birth to increase serum IgG concentration. If identification of a failure of passive transfer does not occur until after 12 hours of age neonates are susceptible to infection, sepsis and death, or may require the administration of plasma transfusions to prevent such events (Francesca et al., 2017; Korosue et al., 2012).

2.2.3 Alpacas

Alpacas are born hypogammaglobulinemic, similar to sheep, cattle and horses and therefore it is critical that they ingest and absorb enough colostral immunoglobulins to achieve adequate serum IgG concentrations (Weaver, Tyler, Scott, et al., 2000; Westrom et al., 2020). There have been no studies in Australia demonstrating the degree of failure of passive transfer present in cria, although Garmendia et al. (1987) found 9% of cria in a Peruvian study to have inadequate concentrations of serum IgG. This study revealed a 77% mortality rate in cria classified with a failure of passive transfer, highlighting the need for further research in the area.

2.3 Direct methods of immunoglobulin analysis

2.3.1 Options

There are several methods of directly assessing IgG concentration including, competitive enzyme-linked immunosorbent assays (ELISA), radial immunodiffusion assays (RID) and immunoturbidimetric (IT) assays.

2.3.2 Radial immunodiffusion assay

Radial immunodiffusion assays (RID) are the gold standard for measuring serum IgG concentration in dairy calves and foals (Bielmann et al., 2010; Elsohaby, Riley, et al., 2019). Radial immunodiffusion assays determine serum IgG concentration by comparing the precipitin ring diameter on an agar plate and the logarithm of the protein concentration which are related in a linear fashion (Figure 2.2). The major disadvantage with RID lies in the time it takes to generate and interpret results, with an 18-24 hour wait time (Davis et al., 2005).

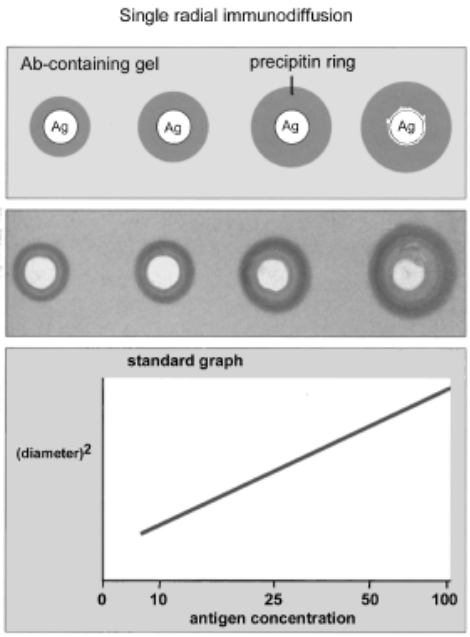

Figure 2.2 Generic RID assay correlation (taken from Figure 29-4, Roitt et al., 1998). Samples of unknown IgG concentration are placed onto antibody-containing gel plates and the precipitin rings that form around each sample measured. The diameter squared of each precipitin ring is then plotted on a standard graph to determine IgG concentration.

2.4 Indirect methods of immunoglobulin analysis

2.4.1 Options

Optical and digital refractometry, total serum protein chelation assays, serum zinc sulfate turbidity test and serum gamma-glutamyl transferase activity (GGT) are indirect methods of assessing IgG concentration in colostrum and blood. Most of these tests are available as commercial kits that can be used on-farm and provide rapid results.

2.4.2 Refractometry

A refractometer measures the total solids in any liquid by measuring the angle of refraction when light enters the liquid (Bielmann et al., 2010; Dairy Australia, 2012). An optical refractometer is a hand-held tool which relies on natural light shining through a sample under a prism, with the operator looking through the eyepiece to identify the level at which the dark and light areas meet on

the scale of the refractometer to determine the level of solids in the sample (Elsohaby, Riley, et al., 2019)(Figure 2.3).

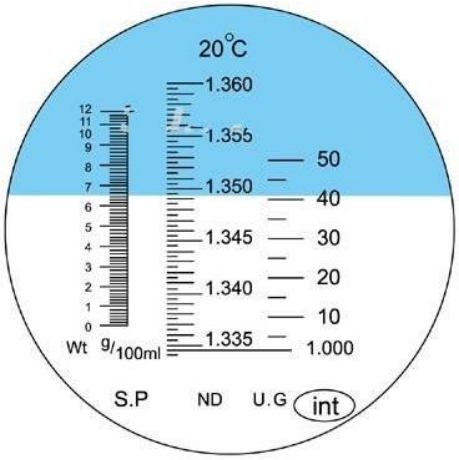

Figure 2.3 Refractometer scale. Total serum protein is determined by reading the scale where the blue and white meet, for example this sample would be approximately 66 g/L (Refractometer Use, 2020). In alpacas total serum protein should be at least 55 g/L (Drew & Fowler, 1995).

A digital refractometer is also a hand-held tool that works by passing an LED light through a sample within a prism. A sensor determines the critical angle of the light and converts the refractive index of the sample, in the case of a Brix refractometer to sucrose concentration, displayed as a percentage (%) Brix (Figure 2.4). The greater the solids in the sample, the more bending of light occurs and the higher the Brix percentage (Bielmann et al., 2010; Dairy Australia, 2012). The optical Brix refractometer works in the same fashion, although the sample is placed onto a prism and the operator observes the percentage by identifying a blue line on a scale, through an eyepiece (Bielmann et al., 2010).

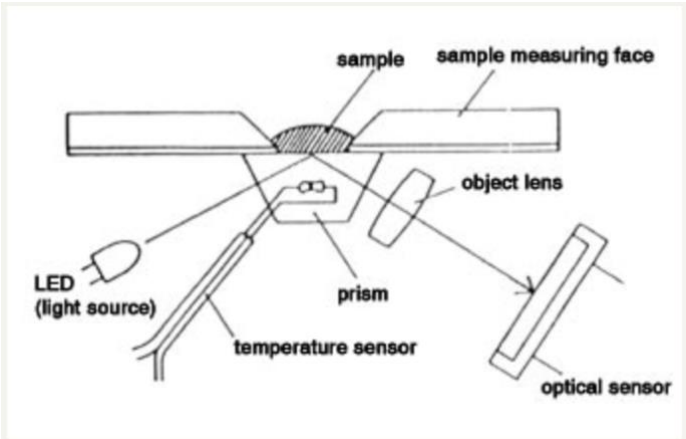

Figure 1.4 How a digital Brix refractometer works, demonstrating the critical angle of light measured by the optical sensor (Refractometers, n.d.).

2.4.3 Refractometry to estimate serum IgG concentrations/FPT in neonates

Total serum protein estimation using refractometry is recommended in dairy systems, as immunoglobulins comprise the majority of the serum protein (after albumin) in neonatal calves and therefore correlate well with serum IgG concentrations up to 9 days of age (Deelen, et al., 2014; Zakian et al., 2018; Wilm et al., 2018). Total serum protein can be measured using a refractometer on-farm and does not require submission of samples to a laboratory, provides results quickly and is inexpensive, making it an attractive tool to estimate serum IgG concentration.

In horses, there are studies that have shown the measurement of total serum protein in foals is less reliable than in calves due to the wide range of total serum protein concentrations observed in neonatal foals (Rumbaugh et al., 1978; Metzger et al., 2006), although more recent research has shown total serum protein estimation using optical refractometry to be accurate (Elsohaby, Riley, et al., 2019).

In the dairy industry, the term failure of passive transfer is used to describe calves with a total serum protein less than 52 g/L (Bielmann et al., 2010). In alpacas, when an endpoint of 45 g/L was used to classify a failure of passive transfer, Weaver et al. (2000) found that 81% of crias were correctly classified, although the specificity was low and the limited research of endpoint values makes it inappropriate to assume the same values used in dairy calves can be used in camelids. Another study suggests that total serum protein should be at least 55 g/L (Drew & Fowler, 1995).

Thornhill et al. (2015) identified that a Brix percentage of 10% provided 100% accuracy in identifying dairy calves (n=48) with serum IgG concentrations above 10 g/L. Zakian et al. (2018) also produced the same accuracy using a Brix percentage of 7.8% in dairy calves, although both studies varied significantly in terms of location of study and ingestion of colostrum. In mares, a Brix percentage of 10% corresponds to an IgG concentration of 8 g/L (Korosue et al., 2012).

2.4.4 Refractometry to estimate IgG concentrations in colostrum

High quality colostrum contains greater than 50 g/L of IgG in cattle and therefore a higher Brix percentage cut-off is used compared to serum IgG analysis (Morrill et al., 2015; Quigley et al., 2013; Zakian et al., 2018). The Brix percentage used to most accurately classify low or high quality colostrum varies throughout the literature in dairy cows from 18% to 23% (Quigley et al., 2013; Morrill et al., 2015; Bartier et al., 2015). An index of 23% Brix is used in mares (F & A, 2019). There has been no research assessing the Brix cut-off value that delineates failure of passive transfer from satisfactory colostrum intake in neonatal alpacas, nor IgG concentrations in alpaca colostrum in Australia.

Table 2.1 Summary of normal ranges for serum and colostral IgG concentration, Brix percentages and total serum protein in bovine, equine, and camelid patients. Normal values for camelids are largely unknown so cut-off values have not yet been established.

Species	Normal range of serum IgG concentration (g/L)	Normal range of colostrum IgG concentration (g/L)	Serum Brix (%)	Colostrum Brix (%)	Total serum protein (g/L)
Bovine	>10	>50-85	7.8-10	18-23	>52
Equine	>8	>50-80	10	23	41-66
Camelid	>9-10	Unknown	Unknown	Unknown	>55

2.5 Factors affecting immunoglobulin concentration

2.5.1 Age of cria at time of blood collection

In alpacas the term failure of passive transfer is used to describe crias with a serum IgG concentration below 9 g/L at 1-3 days of age (Garmendia et al., 1987), although the research to support this value is sparse and varies throughout the literature. More recent studies suggest that IgG concentrations below 10 g/L at 48 hours of age is suggestive of failure of passive transfer in

crias (Pinn et al., 2013; Weaver, Tyler, Scott, et al., 2000), with peak total serum protein and serum IgG concentrations observed at 2-3 days of age (Miller, 2013). Another study states that peak IgG concentration is seen at 24-36 hours of age (Bravo et al., 1997). Weaver et al. (2000) identified the discrepancy in alpacas and explains that serum sample collection should be delayed until 36 hours of age to prevent the risk of incorrectly classifying a cria with adequate serum IgG concentration as having a failure of passive transfer.

2.5.2 Effect of chilling on IgG concentration of colostrum

Refrigeration or a single freeze/thaw cycle of colostrum has no effect on IgG concentration or Brix % compared to fresh colostrum (Morrill et al., 2015; M Tech, 2016; Bielman et al.,2010). The method of thawing (hot water, refrigeration, room temperature or microwave) does not affect IgG concentration of caprine or bovine colostrum (Arguello et al., 2013; M Tech, 2016).

The freezing of serum samples appears to result in a reduction in total protein concentration compared to plasma samples which are more stable through long-term storage (Villarroel et al., 2014). Although this is the case, the variation in total protein was found to be due mainly to variations in albumin not immunoglobulins.

2.5.3 Temperature of sample

It has been suggested that optical and digital Brix refractometry is more accurate when colostrum sample temperatures are uniform due to the effect temperature has on the refractive index (Zakian et al., 2018), although Bielmann et al. (2010) demonstrates that there is no difference in Brix scores at different temperatures. Morrill, et al. (2015) also supports this, with the temperature of colostrum samples not affecting Brix % or refractometry.

2.5.4 Method of analysis

The method used to measure the concentration of serum IgG can alter the interpretation of adequacy of passive transfer of immunity. In one study, sodium sulfate turbidity tests, total serum protein and globulin concentrations were all suitable for assessing passive transfer of immunity in alpaca crias (Weaver et al. 2000). However in another study, Brix refractometry and total serum protein were inaccurate in the assessment of passive transfer in crias (Elsohaby et al. (2017). This suggests that further research is required to determine the accuracy and appropriateness of these tools in alpacas.

2.6 Conclusion

The literature is scant and discrepant regarding optimal methods and cut-off levels that can be used to determine failure of passive transfer in alpacas. The relationship between Brix refractometry and direct measurement of IgG concentration should be explored further in alpacas to optimise cria survival and health.

Chapter 3: Materials and methods

3.1 Aims and hypothesis

The objective of this study was to determine if estimations of IgG concentration in alpaca colostrum and cria serum using indirect methods were correlated to direct measurements of IgG concentration. Optical and digital Brix refractometer readings of colostrum and cria serum were compared to RID assays of alpaca colostrum and cria serum respectively. It was hypothesised that the indirect method of refractometry would provide an on-farm tool to replace laboratory-based RID assays to determine adequacy of passive transfer of immunity in crias.

3.2 Study design

Colostrum samples were obtained from dams within 24 hours of parturition and serum samples were collected from crias at 1-7 days of age for IgG concentration assessment. Approval was obtained from the Animal Care and Ethics Committee and Human Research Ethics Committee of Charles Sturt University (20279 and H20269) prior to the study commencing.

3.3. Alpacas included in the study design

Ten adult females, 8 male crias aged 1-7 days and 2 female crias aged 1-7 days on a Suri alpaca property in the eastern Riverina district of New South Wales were available for sample collection. An additional 3 samples were collected as part of a trial prior to the study, although were not included in the analysis A thorough physical examination was undertaken on dams and crias and animals entered the study based on the inclusion criteria. Any animals that did not meet the inclusion criteria or reference ranges for physical parameters were excluded from the study. There was one hembra and cria excluded from the study.

3.3.2 Colostrum collection

The average age of hembra was 7.4 years, ranging from 3 to 11 years of age. Colostrum was collected at 1-14 hours after parturition. Colostrum samples were collected when the cria-at-foot was less than 24 hours of age (Garmendia et al., 1987; Miller, 2013; Weaver, Tyler, Scott, et al., 2000). Colostrum was collected into a sterile container and stored at -20 °C until IgG analysis was undertaken.

3.3.3 Colostrum laboratory analysis

At the time of analysis the colostrum was thawed in a warm water bath and a Digital Refractometer HI 96811 (Hanna Instruments, Europe, Romania) and E-Line automatic temperature compensation (ATC) range optical Brix refractometer (Bellingham + Stanley, Tunbridge Wells, Kent) was used to measure the total solids in the colostrum. Camelid RID analysis kits (Radial Immunodiffusion Test for Quantification of Camelid IgG in Serum or Plasma®, TRIPPLEJFARMS, Bellingham, Washington, USA) were used for direct IgG assessment. The precipitin ring zone diameters were measured for each sample following incubation and plotted on a standard graph to determine colostrum IgG concentration.

3.3.4 Blood collection and analysis

Blood collection from cria occurred at 1 to 7 days of age, as it has been shown that serum IgG concentrations increase until 24 h of age and then plateau for 7 days before steadily decreasing (Brave et al., 1997; Garmendia et al., 1987; Miller, 2013; Weaver, Tyler, Scott, et al., 2000). Five mL of blood was collected from each cria, of which 4 mL of collected blood was placed into a plain blood tube (BD Vacutainer®, Plymouth, UK) and the remaining 1 mL of blood placed into a

heparin blood tube (Sarstedt Inc, Numbrecht, Germany). Whole blood was allowed to clot at room temperature and then refrigerated before centrifugation at 2500 rpm for 10 minutes (IDEXX, n.d.). Serum was collected from the blood tube and placed into a new plain blood tube and stored at -20°C until analysed for IgG concentration. Heparinised blood was used to measure packed cell volume (PCV) to assess hydration status, with PCV >45% classified as dehydrated. The blood samples were centrifuged at 10,000 rpm for five minutes and the PCV recorded using a PCV reader.

3.3.6 Laboratory analysis

At the time of sample analysis serum was thawed in a 20°C water bath. The digital Brix refractometer was calibrated before each sample analysis in a similar manner to colostrum analysis, and then the serum Brix % recorded. Total serum protein was measured using a clinical refractometer, estimated by observing the reading through the eye piece.

Camelid RID analysis kits were used for direct IgG assessment, as for colostral IgG concentration. The zone diameters of the reference samples were graphed, a line of best fit generated and the unknown serum sample IgG concentrations determined, the same as for colostrum sample analysis (Figure 3.2).

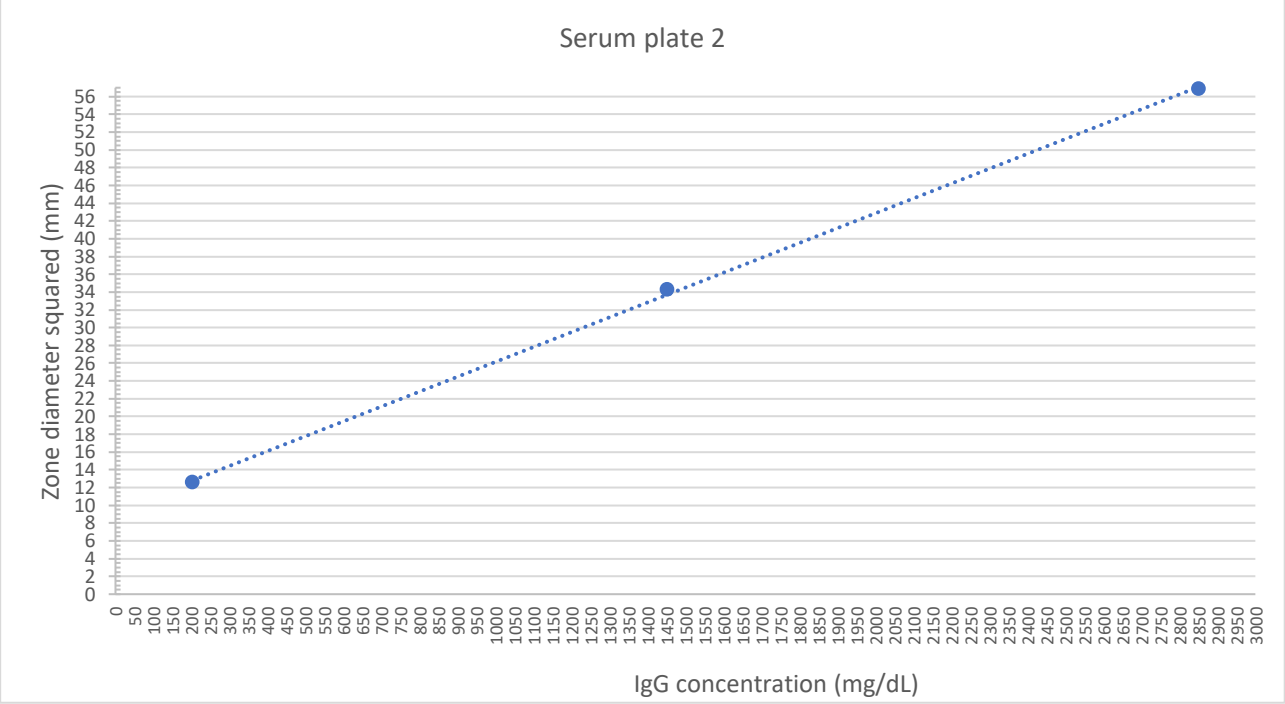

Figure 3.2 An example of a standard graph created for this study.

3.4 Data analysis

Statistical analysis was performed using R: A Language and Environment for Statistical Computing (version 4.0.3) (Team, 2020). Probability (P) values <0.05 were considered statistically significant. Pearson's correlation coefficients of zero were considered as having no linear relationship. Correlation coefficients +/- 30 from zero were considered as having a weak linear relationship. Those > 50 but < 70 were identified as having a moderate correlation and correlation coefficients >70 were considered as having a strong linear relationship. For assessment of sensitivity, cut-off values for Brix refractometry and colostral and serum IgG concentrations were used. For colostrum, high quality was defined as having an IgG concentration >25000 mg/dL and low quality having <25000 mg/dL and a Brix cut-off value of 35% was selected.

For serum, adequate passive transfer of immunity was defined as cria having a serum IgG concentration >1600 mg/dL and inadequate as cria with IgG concentrations <1000 mg/dL. A Brix cut-off value of 7.5% was used.

The optical Brix refractometers upper limit was 32%, so those samples that were read as >32% were assigned a measurement of 32% for statistical analysis.

Chapter 4: Results

4.1 Colostrum IgG concentration

4.1.1 Comparison of Brix refractometry and RID for colostral IgG concentration

The relationship between colostral IgG concentration (determined by RID) and optical Brix refractometry is shown in Figure 4.1.1.

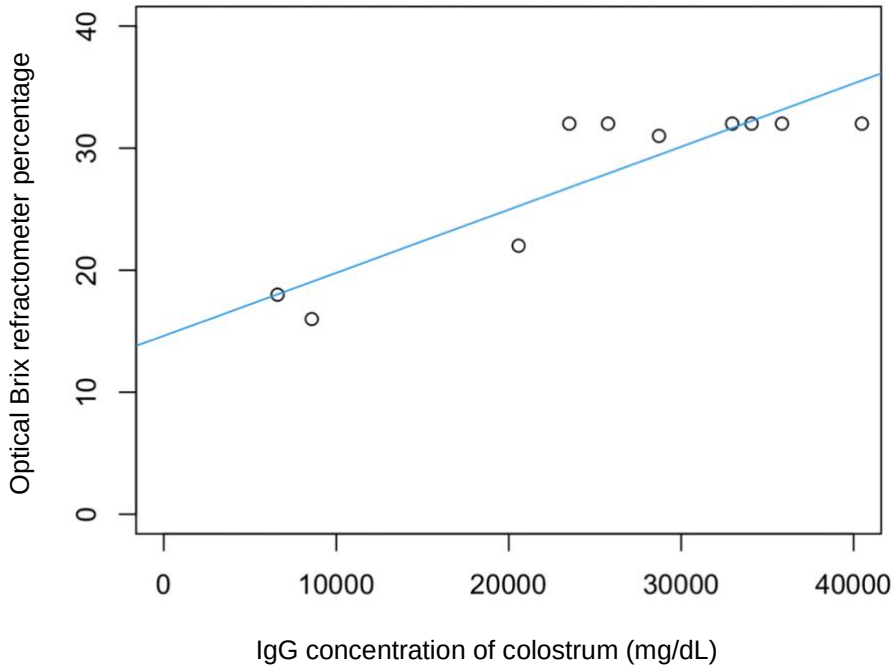

Figure 4.1.1 Relationship between colostrum IgG concentration and optical Brix refractometer for colostrum. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between colostral IgG concentration (determined by RID) and digital Brix refractometry is shown in Figure 4.1.2.

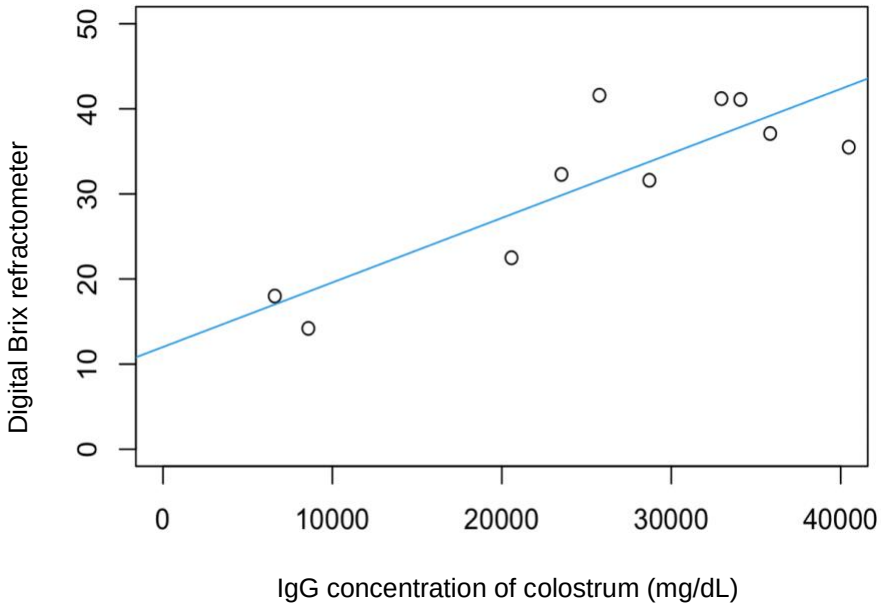

Figure 4.1.2 Relationship between colostrum IgG concentration and digital Brix refractometer for colostrum. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between colostral IgG concentration estimated by optical Brix refractometry and colostral IgG concentration estimated by digital Brix refractometry is shown in Figure 4.1.3.

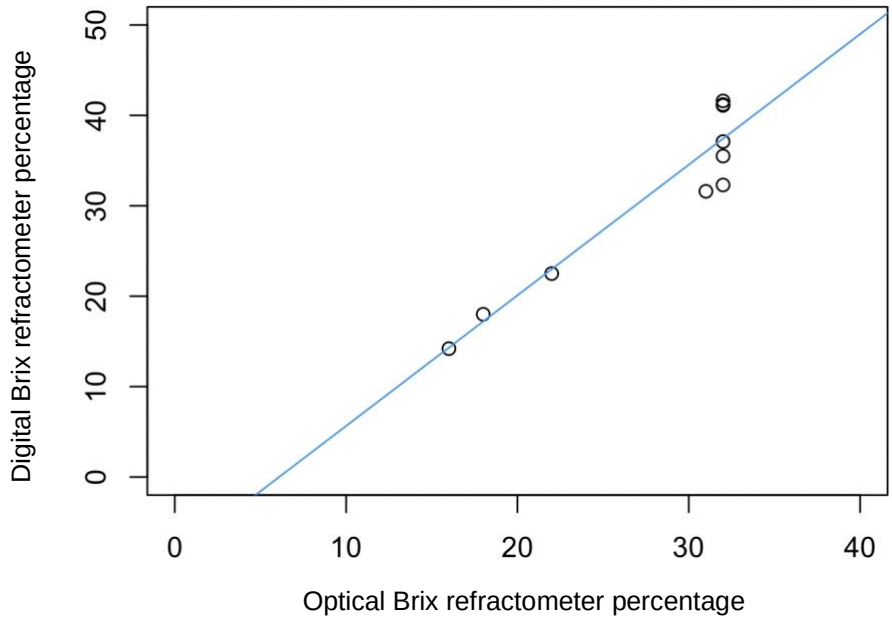

Figure 4.1.3 Relationship between optical and digital Brix refractometer for colostrum. Each data point is represented by a black circle. The blue line is the line of best fit.

4.1.2 Sensitivity of optical and digital Brix refractometers for colostral IgG concentration estimation

The sensitivity of digital and optical Brix refractometers for identifying high- and low-quality colostrum (high = colostrum IgG concentration > 2500mg/dL and low =<2500mg/dL) is shown in Table 4.2 and 4.3 respectively. A Brix cut-off value of 35% was used.

Table 4.2 Sensitivity of the digital Brix refractometer for identifying high (IgG >25000mg/dL) and low (IgG < 25000mg/dL) quality colostrum. 'Yes' represents correct identification and 'No' represents incorrect identification.

		Colostrum IgG	
Digital Brix		High	Low
	No	1	4
	Yes	5	0

Table 4.3 Sensitivity of optical Brix refractometer for identifying high (IgG >25000mg/dL) and low (IgG < 25000mg/dL) quality colostrum. 'Yes' represents correct identification and 'No' represents incorrect identification.

		Colostrum IgG	
Optical Brix		High	Low
	No	0	3
	Yes	6	1

4.2 Serum IgG concentration

4.2.1 Comparison of Brix refractometry, total serum protein and RID for serum IgG concentration

A moderate linear correlation was present between RID-determined serum and colostral IgG concentration (Figure 4.2.1).

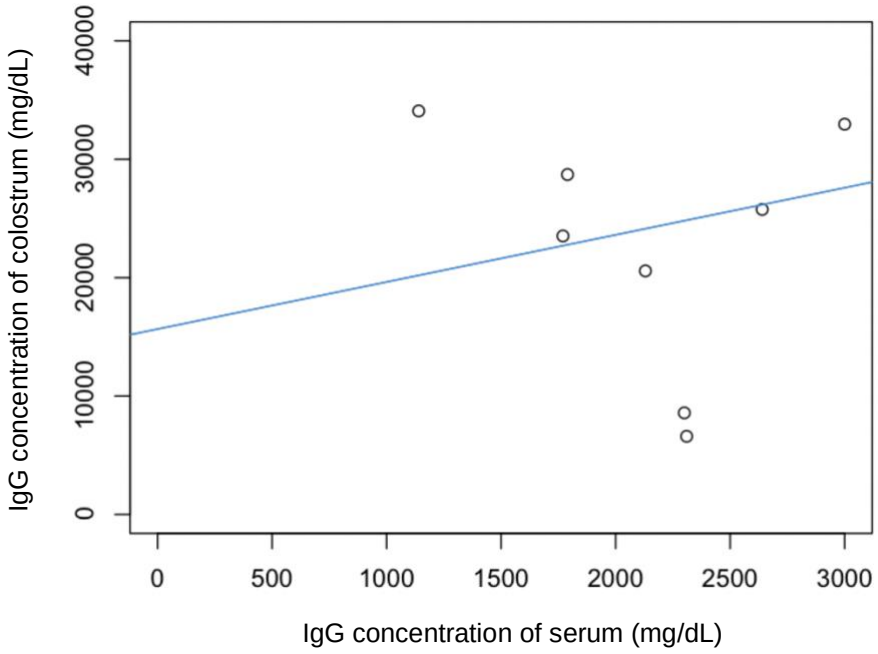

Figure 4.2.1 Relationship between serum and colostrum IgG concentration, determined by RID. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between serum IgG concentration (determined by RID) and optical Brix refractometry is shown in Figure 4.2.2.

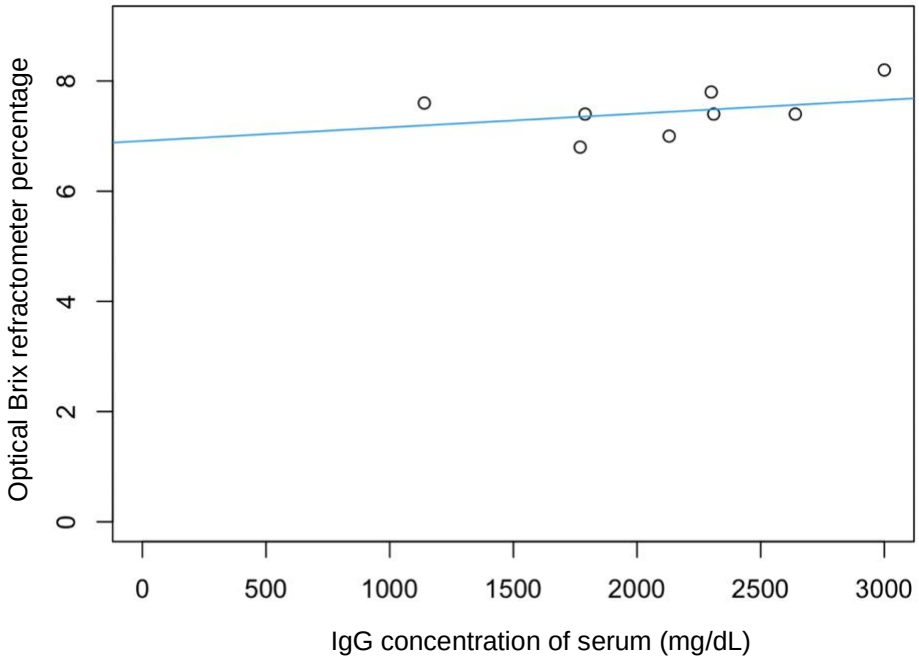

Figure 4.2.2 Relationship between serum IgG concentration (determined by RID) and optical Brix refractometer for serum. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between serum IgG concentration (determined by RID) and digital Brix refractometry is shown in Figure 4.2.3.

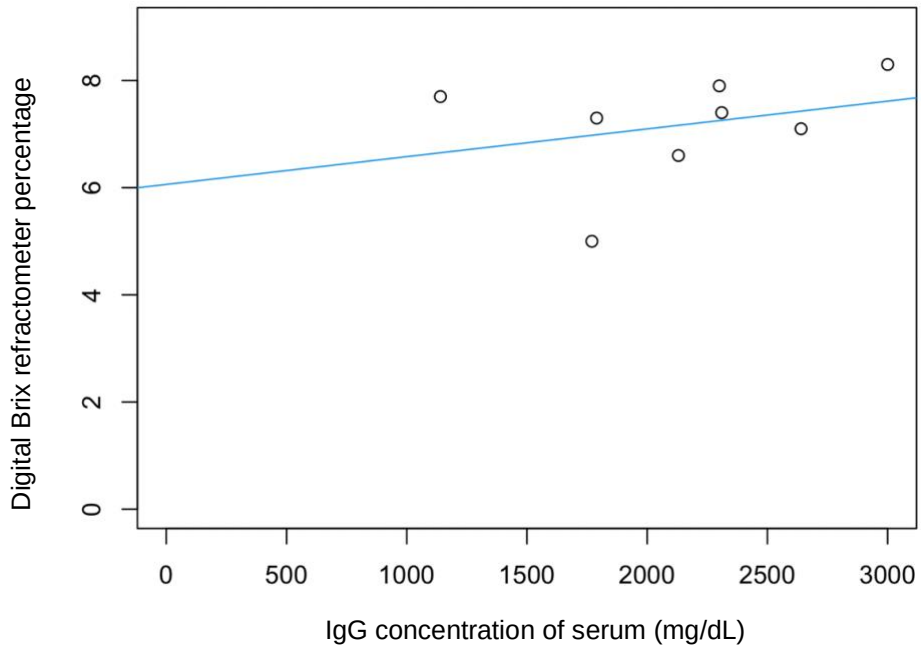

Figure 4.2.3 Relationship between serum IgG concentration and digital Brix refractometer for serum. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between serum IgG concentration (determined by RID) and total serum protein is shown in Figure 4.2.4.

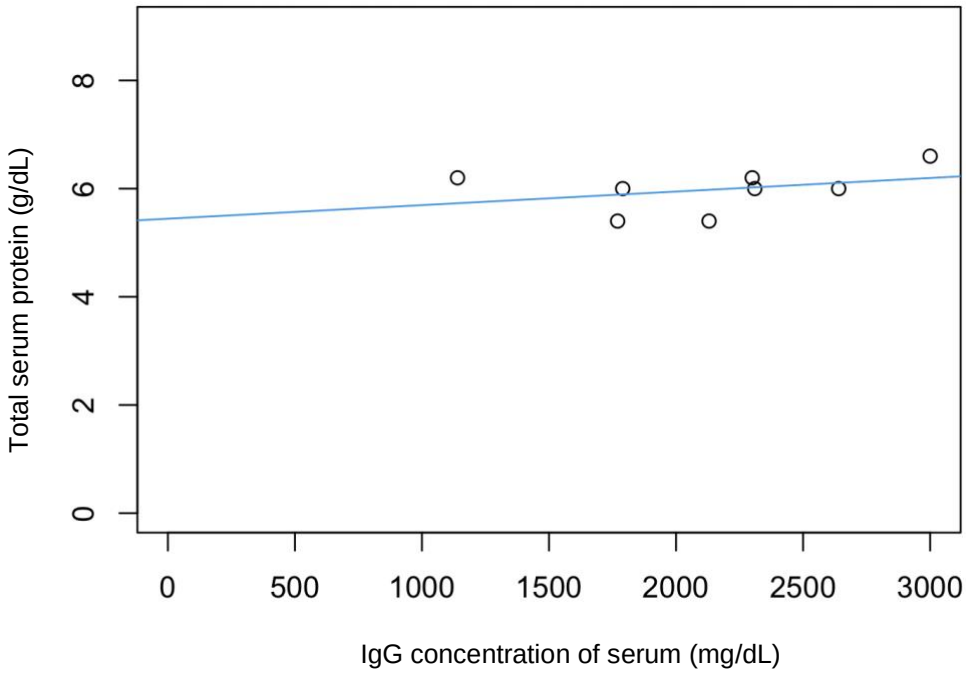

Figure 4.2.4 Relationship between serum IgG concentration and total serum protein. Each data point is represented by a black circle. The blue line is the line of best fit.

The relationship between optical and digital Brix refractometry for estimation of serum IgG concentration is shown in Figure 4.2.5.

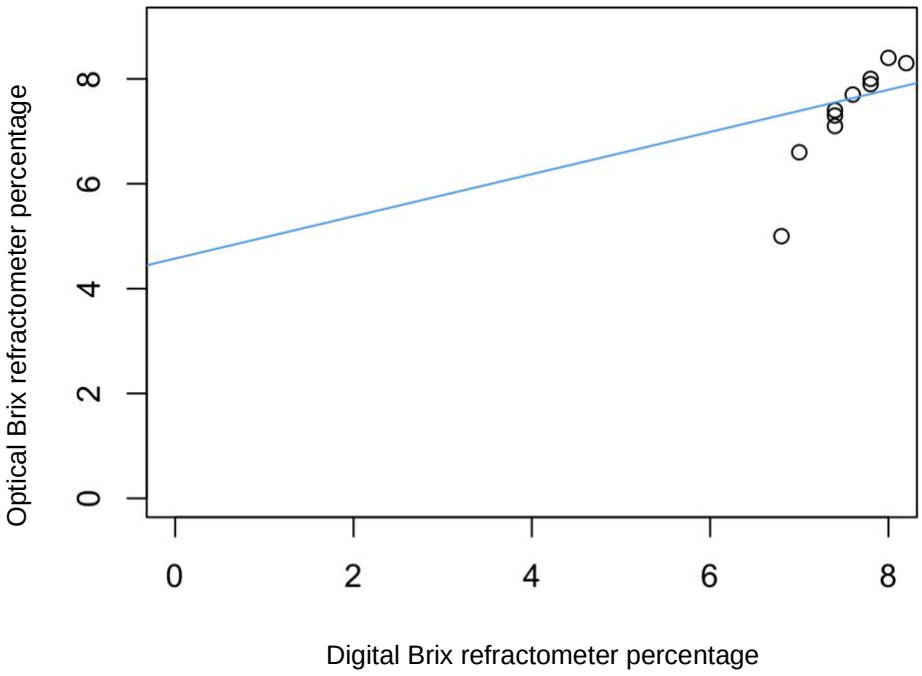

Figure 4.2.5 Relationship between optical and digital Brix refractometer for serum. Each data point is represented by a black circle. The blue line is the line of best fit,

4.2.2 Sensitivity of optical and digital Brix refractometers for serum IgG concentration estimation

The sensitivity of digital and optical Brix refractometers for identifying adequate and inadequate passive transfer of immunity are shown in Table 4.5 and Table 4.6, respectively (adequate classified as cria with serum IgG concentration >1600mg/dL and inadequate as cria with serum IgG concentration = <1000mg/dL). A Brix cut-off value of 7.5% was used.

Table 4.5 Sensitivity of digital Brix refractometer for identifying adequate (IgG >1600mg/dL) and inadequate (IgG < 1000mg/dL) passive transfer of immunity. 'Yes' represents correct identification and 'No' represents incorrect identification.

		Serum IgG	
Digital Brix		High	Low
	No	4	1
	Yes	5	0

Table 4.6 Sensitivity of optical Brix refractometer for identifying adequate (IgG >1600mg/dL) and inadequate (IgG < 1000mg/dL) passive transfer of immunity. 'Yes' represents correct identification and 'No' represents incorrect identification.

		Serum IgG	
Optical Brix		High	Low
	No	4	1
	Yes	5	0

Chapter 5: Discussion

5.1 Interpretation of results

This study supported the hypotheses that on-farm tools such as Brix refractometry and total serum protein can be used to estimate colostral and serum IgG in alpacas. Optical and digital Brix refractometry was found to be an appropriate and accurate tool for assessing colostral IgG concentration, and optical and digital Brix refractometry and total serum protein were also accepted as usable estimates of serum IgG concentration in alpacas.

The assessment of optical and digital Brix refractometry, compared to RID analysis for colostrum resulted in a strong correlation with statistical significance and a high sensitivity for both indirect tools. To the authors knowledge there are no published studies describing the use of Brix refractometry in assessing colostral IgG in alpacas, although similar studies in dairy cattle and horses demonstrate similar outcomes to the current project (Morrill, et al., 2015; Bartier, et al., 2015). The results of the current project and the aforementioned studies suggest that even though there is variation, optical and digital Brix refractometers are accurate in estimating colostral IgG concentration in cattle, horses and alpacas.

The assessment of optical and digital Brix refractometry and total serum protein, compared to RID analysis for serum resulted in a moderate correlation and high sensitivity of all indirect tools. Weaver et al. (2000) provides a similar conclusion as the current study, that the use of total serum protein as an estimation of serum IgG in alpaca cria requires further research to determine the endpoint values for camelids.

This study also aimed to determine if serum IgG and colostral IgG were correlated in alpacas. The results showed a moderate correlation between serum and colostral IgG, although did not achieve

statistical significance, likely due to the small study population. In hypogammaglobulinaemic cria, ingestion of colostrum containing higher IgG levels is expected to see a greater increase in serum IgG than ingesting colostrum containing lower IgG levels, as observed in this study.

Dam parity and dam age did not have any effect on colostral IgG concentration, although assessment of these parameters was not statistically significant, likely due to the small study population. This result varies from studies in dairy and beef cattle, with McGee & Earley (2019) and Bartier et al. (2015) suggesting that passive transfer of immunity is affected by parity and that increased parity results in higher colostral IgG concentration.

The moderate correlation and lack of statistical significance between cria age and serum IgG concentration suggests that cria age may affect serum IgG concentration although the small sample size and limited numbers of animals in each age category means further research is likely required to assess the impact of cria age on serum IgG concentration. A weak correlation between cria weight and serum IgG concentration was found.

The use of optical and digital Brix refractometry in assessing colostral IgG concentration, and optical and digital Brix refractometry and total serum protein in assessing serum IgG concentration means that alpaca owners can assess the adequacy of passive transfer of immunity soon after birth. This will enable intervention measures to be implemented early if required to improve the survivability of cria.

5.2 Limitations of study

The use of optical and digital Brix refractometers for the assessment of serum IgG concentration has limitations on-farm, with training of on-farm staff required to enable blood samples to be obtained. The use of optical and digital Brix refractometry for the assessment of IgG concentration

in alpaca colostrum requires only hand-milking into a sterile jar which is a simple procedure already undertaken by many alpaca breeders when assessing colostrum flow. Although this is the case the volume of alpaca colostrum available for collection is limited compared to dairy cows and milking can be difficult.

Accurate dehydration assessment in cria was challenging due to the lack of clinical signs presenting in mild to moderate cases. The use of Brix refractometry must take into account the clinical status of the cria, with dehydrated or sick cria likely to produce inaccurate results. When an animal is dehydrated the blood components, including protein become concentrated and results in a higher Brix % which means these animals will be incorrectly classified as having an adequate passive transfer of immunity. This also applies for total serum protein, with dehydrated animals likely to have higher protein concentration due to the concentrating effects of dehydration which leads to greater refraction of light in the serum on refractometry (Buczinski et al., 2018). If owners are concerned that dehydration may be playing a role in high refractometer values, PCV and urinalysis can be performed by a veterinarian to confirm dehydration.

Accessibility of Camelid RID kits is limited in Australia, with only a single supplier able to be found and the timeframe of delivery being several weeks. This means that the use of these kits requires at least 3-4 weeks of planning prior to the arrival of cria and until the use of these kits becomes more common it is unlikely that colostral or serum IgG analysis via RID is feasible in Australia.

On-farm IgG concentration analysis requires sample collection within 1-7 days of birth, as readings outside this time frame are often inaccurate. This limitation means owners need to know the day of birth to be able to interpret Brix scores. All registered alpacas in Australia are required to have their date of birth recorded on a national database, so it can be assumed that many alpaca owners have

adequate records and monitoring procedures in place to estimate date of birth within a 24-hour period.

The average age of alpacas in this study was 7.4 years and average parity was 1.6. Alpacas are known to have optimum fertility at a younger age, as well as a high prevalence of pseudopregnancies (San-Martin et al., 1968) which limited the quantity of samples available for collection in this study. An additional limitation was that the majority of the adult females sampled were maidens and a reasonable portion of females that spat-off positive did not birth a live cria. The use of ultrasonography to confirm pregnancies may have enabled greater accuracy in the prediction of parturition in this project. The unexpected number of abortions and still-borns on this property may have also been attributed to ongoing drought and extreme heat during the first batch of collections, and then floods and cold weather during the second batch.

The unexpected limitation of COVID-19 restrictions meant that sample collection could only occur in New South Wales and the interpretation of colostral and serum IgG concentrations could not include other regions that may produce different results. This study also only assessed the use of tools for assessing passive transfer of immunity in the Suri breed, not Huacaya's, so it is not known whether differences exist.

5.3 Future opportunities

The hypotheses have been accepted, that Brix refractometry and total serum protein can be used to estimate colostral and serum IgG concentration in alpacas, although the concentration of IgG that provides adequate protection in alpacas is unknown and provides an area for further research.

The use of plasma transfusions to increase serum IgG is supported in the equine industry (Korosue et al., 2012), although the literature in the dairy industry is limited in its practical use as most

studies have assessed the effectiveness of plasma transfusions on experimentally colostrum-deprived calves (Boccardo et al., 2016; Pipkin et al., 2015). Additionally, there is a lack of agreement in the success of plasma transfusions in calves. The literature is very limited in the alpaca industry and the few studies that do recommend the administration of camelid plasma at 20-40 mL/kg have no clinical research to assess its effectiveness (Paxson et al., 2008; Wernery, 2001).

In alpacas the serum IgG concentration threshold for diagnosis of failure of passive transfer is inconsistent throughout the literature, with 9 g/L at 1-3 days of age and 10 g/L at 48 h of age being suggested (Garmendia et al., 1987; Pinn et al., 2013). The literature is sparse and requires further research to determine the appropriate cut-off values for direct and indirect methods of colostral and serum IgG analysis, particularly in Australian alpacas. Without consistent cut-off values the widespread use of on-farm tools will be difficult and confusing for many owners and less likely to be accurate. The lack of research in alpaca serum and colostral IgG concentration opens many opportunities for further research, particularly in Australia where camelids live in conditions that vary enormously from overseas herds.

Chapter 6: Conclusion

The results of this study demonstrate that optical and digital Brix refractometry are accurate tools to estimate colostral IgG concentration in alpacas, and that optical and digital Brix refractometry and total serum protein are accurate tools in estimating serum IgG concentration in alpaca cria. The study also found that colostral IgG and serum IgG concentrations are moderately correlated, although dam parity and age and cria weight appear not to have significant effects on passive transfer of immunity. Published literature in dairy and equine industries accept Brix refractometry as reliable and accurate in assessment of failure of passive transfer, and total serum protein as accurate in diary calves but not foals (Thornhill et al., 2015; Metzger et al., 2006). Repetition of this study with alterations including an increased study population to provide greater statistical power and inclusion of greater variation in location, age, parity and breed to allow adequate comparison between possible influencing factors could improve the accuracy and significance of results to represent the Australian alpaca herd. Limitations that were uncontrolled by the study design, including lack of appropriate cut-off values for Brix refractometry and total serum protein can be overcome through further research in the future.

Future research of the reference intervals for colostral and serum IgG concentration and PCV in alpacas would provide essential information required for the management of neonatal alpacas and use of on-farm tools such as Brix refractometry and total serum protein. Additionally, the intervention measures available for cria with inadequate passive transfer of immunity such as plasma could be studied to determine their effectiveness in improving survivability. In conclusion, Brix refractometry and total serum protein can be considered valuable additions to alpaca neonatal care on-farm and may play an important role in detecting a failure of passive transfer with further research.

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